

Original Research Article

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Compatibility of Fungicides on Sporulation and Growth of *Trichoderma viride* with Treated Seeds

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ABSTRACT

Trichoderma spp. are tolerant and compatible with fungicides, biofertilizers and other bio pesticides. Integrating fungicide resistant antagonists with suitable fungicide treatment has importance in the frame work of integrated disease management. Incorporation of natural products provides a viable solution to the environmental problems caused by synthetic pesticides. Identification of these compounds and their further testing may be an effective approach to minimize the use of hazardous chemicals. With the above mentioned points the present research work was conducted to assess the sporulation and growth of native isolate of *Trichoderma viride* on fungicide treated seeds. The research work was carried out by isolation and identification of native isolate of *T. viride*, sporulation of *T. viride* on fungicide amended medium and growth of *T. viride* on fungicide treated seeds. Various fungicides viz., carbendazim (0.1, 0.2 and 0.3 per cent), copper oxy chloride (0.2, 0.3 and 0.4 per cent), thiophanate methyl (0.05, 0.1 and 0.2 per cent), benomyl (0.05, 0.1 and 0.2 per cent) and sodium hypochlorite (5, 10 and 15 per cent) were used in this experiment. Sporulation of *T. viride* was observed in all fungicides except in thiophanate methyl and sodium hypochlorite at higher concentrations. Copper oxychloride showed the highest sporulation of 185×10^5 spores / ml and benomyl recorded 35×10^5 spores / ml as least. Mycelial growth of *T. viride* was measured as 2.37 mm in sodium hypochlorite and 2.27 mm in copper oxy chloride treated seeds around the *Colletotrichum capsici* infected seeds as against 3.50 mm in control.

Keywords

Trichoderma viride,
Sporulation,
Growth, Seeds,
Fungicides and
Sodium
hypochlorite

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Introduction

Development of effective disease management programme requires the compatibility of potential bio agents with commonly used fungicides. Combination of chemicals and compatible bio agents is an

IDM strategy to protect the seeds and seedlings from soil and seed borne fungi (Dubey and Patil, 2001). Combining antagonists with synthetic and non-synthetic chemicals eliminates the chance of resistance development and reduces the fungicide application (Ashwani Tapwal *et al.*, 2012). In

recent years, both in developed and developing countries the use of hazardous fungicides for controlling plant diseases proven to cause adverse environmental effects and results in health hazards to human as well as other organisms including beneficial and natural enemies. Natural control of several phytopathogens was observed based on the presence of suppressive soils where several biocontrol microorganisms were detected such as *Trichoderma*, *Pseudomonas* and *Bacillus* (Huang *et al.*, 2005). Integration of biocontrol agents with other control practice might be effective and provide acceptable levels of disease control with reduced level of chemicals. With the above aspects this work was carried out to study the compatible nature of commonly used fungicides with optimum, lower and higher concentrations on sporulation and growth of native isolate of *T.viride*.

Materials and Methods

Fungicides on sporulation of *T.viride*

Sporulation of *T. viride* in different concentrations of various fungicides was assessed by using haemocytometer. The fungicides *viz.*, carbendazim (Bavistin 50 % WP) 0.1, 0.2 and 0.3 per cent, copper oxy chloride (Fytolan 50% WP) 0.2, 0.3 and 0.4 per cent, thiophanate methyl (Hexamar 70 % WP) 0.05, 0.1 and 0.2 per cent, benomyl (Benofit 50 % WP) 0.05, 0.1 and 0.2 per cent and sodium hypochlorite (Bleaching powder 3 – 8 %) 5, 10 and 15 per cent) were used in this experiment. In 100 ml of sterilized Czapek's broth in 250 ml conical flasks, different concentrations of above fungicides were added Control flasks were maintained without fungicides.

Three replications were used for each treatment. The 9 mm disc of three days old culture of *T. viride* was inoculated in all flasks

and incubated at room temperature for 15 days. At the end of incubation period, the contents were mixed thoroughly to dislodge the spores. After through shaking a drop of the filtrate was placed in a haemocytometer and the number of spores per unit was determined under microscope as per the procedure to count the cells or spores by using haemocytometer. Calculation was made by using the formula

$$\text{Cells (or) spores / ml} = \frac{\text{Average number of spores counted (Medium Square)}}{\text{Volume of fluid (ml)}}$$

The sporulation of the *T. viride* was expressed as $\times 10^5$

Fungicides and *T. viride* on seed borne pathogen – *Colletotrichum capsici*

Supernatant metabolites of *T. viride* were collected from Czapek's broth inoculated with *T. viride* by filtering with Whatman No.1 filter paper. One ml of the *T. viride* metabolite was poured in to sterilized Petri plate and then with Czapek's medium. After solidification of the medium, various fungicides treated seeds of chillies were placed individually at the rate of 25 seeds per plate after shade drying. Chillies seeds were collected from *Colletotrichum capsici* infected fruits. For each concentration of different fungicides *viz.*, carbendazim (Bavistin 50 % WP) 0.1, 0.2 and 0.3 per cent, copper oxy chloride (Fytolan 50% WP) 0.2, 0.3 and 0.4 per cent, thiophanate methyl (Hexamar 70 % WP) 0.05, 0.1 and 0.2 per cent, benomyl (Benofit 50 % WP) 0.05, 0.1 and 0.2 per cent and sodium hypochlorite (Bleaching powder 3 – 8 %) 5, 10 and 15 per cent, three replications were maintained. In control plates seeds were not treated with fungicides. Plates were incubated at room temperature ($30 \pm 2^\circ \text{C}$) and observed the growth of *T. viride* around the seeds by compared with control plates.

Statistical analysis

Data of the experiments were analysed by Completely Randomized Block Design (CRD) using data entry module for Ag Res Statistical Software© 1994 Pascal International Software Solutions, version 3.01 for data entry and version 7.01 for analysis.

Results and Discussion

Sporulation of *T. viride* and its growth on fungicides treated seeds on the basis of compatibility was studied in this research work. The native isolate of *T. viride* was tested against various fungicides with different concentrations under *in vitro* condition. Fungicides were used as lower,

actual and higher concentrations for tolerance effect of *T. viride* sporulation and its growth on treated seeds.

Sporulation of *T.viride* on different fungicides

Experiment was conducted to study the influence of fungicides on sporulation of *T. viride* and the results were summarized in Table 1. Sporulation was observed in all fungicides and lower concentration of sodium hypochlorite except the higher concentrations of thiophanate methyl (0.20 per cent) and sodium hypochlorite (10.00 and 15.00 per cent). Drastic reduction of spores was observed in increasing concentrations of each fungicide.

Table.1 Effect of different fungicides on sporulation of *Trichoderma viride*

S.No.	Fungicides	Concentration (%)	Sporulation (x10 ⁵)
1.	Carbendazim	0.10	155 d
		0.20	148 e
		0.30	143 g
2.	Copper oxy chloride	0.20	185 b
		0.30	183 c
		0.40	143 g
3.	Thiophanate methyl	0.05	138 h
		0.10	95 k
		0.20	-
4.	Benomyl	0.05	145 f
		0.10	125 j
		0.20	35 l
5.	Sodium hypochlorite	5.00	135 i
		10.00	-
		15.00	-
6.	Control	-	233 a

S.E. = 0.64 C.D. (0.05) = 1.31

Table.2 Growth of *Trichoderma viride* in fungicide treated seeds

S.No.	Fungicides	Concentration (%)	Growth of <i>T. viride</i> (mm)
1.	Carbendazim	0.10	2.05 cdef
		0.20	2.03 cdef
		0.30	1.18 h
2.	Copper oxy chloride	0.20	2.27 bc
		0.30	2.09 bc
		0.40	1.88 ef
3.	Thiophanate methyl	0.05	1.89 ef
		0.10	1.83 ef
		0.20	1.79 fg
4.	Benomyl	0.05	1.98 def
		0.10	1.54 g
		0.20	0.68 i
5.	Sodium hypochlorite	5.00	2.37 b
		10.00	2.21 bcd
		15.00	1.51 g
6.	Control	-	3.50 a

S.E. = 0.14

C.D. (0.05) = 0.28

Among the fungicides, copper oxychloride showed the highest sporulation of 185×10^5 spores / ml. Benomyl at 0.20 per cent concentration had the least of 35×10^5 spores / ml.

These results were in accordance with the report of Shukla (2011) that *T. viride* with fungicides, fytolan showed the highest sporulation and bavistin completely inhibited the sporulation. These results also showed the same trend of highest sporulation in copper oxychloride and least at benomyl. Mc Lean *et al.*, (2001) suggested that in many fungicidal treatment there was an initial decline in *Trichoderma* colony forming unit counts and then gradual recovery over time. It is possible that the fungicides reduced the germination capability of the initial spore inoculum but subsequently, the germinated spores established and sporulated in the soil to bring the cfu counts back up to $10^4 - 10^5$ g/ soil.

Growth of *T. viride* in fungicide treated seeds

In this experiment the growth of *T. viride* around the fungicide treated seeds were observed and the results were given in Table 2. Fungicide treated seeds placed on the *T. viride* amended medium showed significant differences among the fungicides compared to control.

Radial growth of *T. viride* was measured around the seeds of control showed the maximum of 3.50 mm followed by 2.37 mm in sodium hypochlorite, 2.27 mm in copper oxy chloride and 2.05 mm in carbendazim.

In all the fungicides there was no significant difference in growth among the concentrations of each fungicide. Benomyl treated seeds showed higher inhibition of *T. viride* growth (0.68 mm at 0.20 per cent)

Results of this study contradictory with Mc Lean *et al.*, (2001). They reported that *T. harzianum* C52 strain applied at planting with captan and/ or benomyl treated onion seeds provided the control of other seedling diseases.

In conclusion the sporulation of *T. viride* was observed in all the fungicides except the higher concentrations of thiophanate methyl and sodium hypochlorite in which no spores were formed. Highest sporulation was of *T. viride* were obtained in copper oxy chloride and least in benomyl. Radial growth of *T. viride* was measured around the fungicide treated and untreated seeds. Maximum growth was measured around control (untreated seeds) followed by sodium hypochlorite and copper oxychloride. All the fungicides and *T. viride* inhibited the conidia of *Colletotrichum capsici* in the infected seeds and then *T. viride* overgrew around the chillies seeds. Seed borne *Colletotrichum capsici* in chillies was inhibited by fungicides and *T. viride* in combination.

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